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Patentanmeldung Nr. Patent application No. Demande de brevet n°

02079232.1

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Yamanouchi Europe B.V.
Elisabethhof 19
2353 EW Leiderdorp
PAYS-BAS

Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se référer à la description.)

Glucose-based compounds with affinity to P-Selectin

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Title: Glucose-Based Compounds with Affinity to P-Selectin

Description

FIELD OF THE INVENTION

The present invention relates to sugar-derived compounds which
5 bind selectively to the adhesion molecule human P-selectin, to the use of such
compounds in therapeutic or diagnostic methods and in pharmaceutical
compositions, to conjugates, pharmaceutical carriers and drug delivery
systems comprising said compounds, and to a method for determining whether
a compound is capable of binding to P-selectin.

10

BACKGROUND OF THE INVENTION

In recent years, cell surface adhesion molecules have become
recognized as key mediators in numerous cellular processes including cell
growth, differentiation, immune cell transmigration and response, and cancer
15 metastasis.

Four major categories of adhesion molecules have been identified:
the immunoglobulin superfamily cell adhesion molecules (CAMs), cadherins,
integrins, and selectins.

The selectins represent a family of presently three
20 transmembraneous, carbohydrate-binding glycoproteins: "endothelial" E-
selectin, "leukocyte" L-selectin, and "platelet" P-selectin. All three selectins are
divalent cation (e.g. calcium) dependent and possess an extracellular domain
with a carbohydrate recognition motif, an epidermal growth factor-like motif,
and some smaller domains related to complement-regulatory proteins.

25

Human P-selectin (also referred to as GMP-140, LECAM-3,
PADGEM, CD62, and CD62P) is expressed by platelets and endothelial cells.
When expressed on the surfaces of these cells, its most notable effect is the

slowing of leukocytes as these leave the capillaries and enter the postcapillary venules, the latter representing the major site of leukocyte-endothelium adhesion. The slowing process is observed as leukocyte rolling, signifying an initial adhesion with relatively low affinity. The firm adhesion of rolling
 5 leukocytes is primarily mediated by integrins.

In endothelial cells, P-selectin is stored on Weibel-Palade bodies; in platelets, it is found in the α -granules. Following activation, P-selectin is mobilized to the cell surfaces within a few minutes in response to a variety of inflammatory or thrombogenic agents. The endothelial P-selectin's primarily
 10 function is to recruit leukocytes into postcapillary venules, while platelet P-selectin also results in the formation of thrombi. One of the presently known natural ligands of P-selectin is PSGL-1 (P-selectin glycoprotein ligand-1), a 160 kDa sialoprotein expressed on the surface of leukocytes where it is concentrated at the uropod. More detailed descriptions of the structure and
 15 functions of P-selectin are found in numerous publications, such as J. Panes, *Pathophysiology* 5, 271 (1999); F. Chamoun *et al.*, *Frontiers in Bioscience* 5, e103 (Nov. 1, 2000); S.-I. Hayachi, *Circulation* 102, 1710 (2000).

Inflammation and inflammatory processes play a major role in the pathophysiology of numerous diseases and conditions. Conditions of the brain
 20 in which increased selectin levels were found, and which may therefore involve selectin-mediated pathophysiological events, include severe traumatic brain injury, relapsing-remitting multiple sclerosis, cerebral artery occlusion, ischemia, and stroke. Conditions of the heart in which selectins are suggested to play a role include acute myocardial infarct, arterial injury, such as
 25 produced by angioplasty, and ischemia. Similarly, selectins are involved in conditions of the kidneys, such as renal injury from ischemia and reperfusion, and renal failure. Furthermore, selectins appear to play a role in organ
transplant rejection, cold ischemia, hemorrhagic shock, septic shock, tumor
 metastasis, chronic inflammation, rheumatoid arthritis, inflammatory bowel

disease, atherosclerosis, restenosis, angiogenesis, disseminated intravascular coagulation, adult respiratory stress syndrome, and circulatory shock.

Thus, it would seem feasible to improve these and other conditions involving the activation of endothelial cells and leukocytes and specifically the mobilization and expression of P-selectin by specifically interrupting the P-selectin cascades. This can be done, for instance, by the administration of ligands which selectively bind to human P-selectin, but which do not possess its bioactivity. By this method, mobilized P-selectin could be inactivated and leukocyte-induced tissue damage prevented. Potentially, the same effect could be achieved by gene therapy, provided the P-selectin ligand or antagonist is a peptide or modified peptide. According to this method, somatic cells of a person in need of the therapy would be transfected with an expression vector carrying a DNA sequence encoding a P-selectin antagonist.

P-selectin ligands or antagonists may also be used for the prevention of diseases and conditions described above. Furthermore, such ligands may also be useful in the *in vivo* or *in vitro* diagnosis of these diseases.

Various attempts have been made in recent years to identify or create such selective ligands to P-selectin. So far, a number of substances were tested, but no clinical studies have yet provided conclusive evidence that any of these compounds produce the desired clinical effects while being tolerable in terms of side effects.

For instance, antibodies to P-selectin that were produced and tested in animal models, were found to protect kidneys from ischemic-reperfusion injury (H. C. Rabb *et al.*, JASN 5, 907, 1997; US-A-6,033,667). In another study, a recombinant soluble form of P-selectin glycoprotein ligand-1 (rPSGL-Ig) was used to inhibit thrombosis in cats (M. J. Eppihimer *et al.*, Arteriosclerosis, Thrombosis, and Vascular Biology 20, 2483, 2000). WO-A-99/41363 discloses podocalyxin-like proteins that bind to selectins. WO-A-00/41711 describes various smaller peptides or peptide sequences that bind to

members of the human selectin family; most of the sequences comprise one or more units of leucine or isoleucine.

As another approach to inhibit the P-selectin cascade, various peptides derived from the lectin domain of the selectin family were found to
5 inhibit neutrophil adhesion to P-selectin (*e.g.* US-A-6,111,065 and US-A-5,916,876); these peptides probably bind to P-selectin receptors on leukocytes.

In WO-A-94/05269, peptides are described which inhibit binding of selectins such as P-selectin, E-selectin and L-selectin. These peptides have as
10 their core region portions of the 11-18 amino acid sequence of P-selectin, E-selectin or L-selectin. Further, WO-A-95/31210 relates to peptides and compounds that bind selectins including endothelium leukocyte adhesion molecule 1 (ELAM-1). These peptides are used for blocking adhesion of leukocytes to the selectins, *i.e.* especially E-selectin, but also P-selectin or L-selectin, for the purpose of inhibiting inflammation.

15 In Applicant's pending patent applications PCT/NL02/00566 and European application no. 02078308.0, novel compounds are described with peptidic core regions and certain consensus motifs which are essential for recognizing P-selectin. In contrast to most other known substances which have an affinity to the selectins, these compounds possess a high degree of P-
20 selectin specificity. Little is yet known about the stability of these compounds *in vitro* and *in vivo*, and as in the case of most therapeutic or diagnostic peptides, in order to become bioavailable it is preferred that these compounds be injected. Most patients, however, prefer non-parenteral routes of administration, especially for chronic therapies.

25 Apart from the peptide approach, the fact that the selectins recognize sialyl Lewis sugar X (sLeX) has also led to a number of efforts towards the development of sugar-based ligands having selectin-binding activity. For example, US-A-5,750,508 discloses synthetical ligands based on
30 fucose and sialic acid, wherein the fucose and sialic acid moieties are separated by non-carbohydrate linkers. WO-A-96/09309 discloses oligosaccharide

structures that are potential ligands to E- and P-selectin. WO-A-98/06730 describes modified oligosaccharides which are derivatives of sLeX, in which the neuraminic acid residue and the N-acetylglucosamine monomer have been replaced by various substituents. US-A-5,830,871 discloses sugar-based
5 selectin inhibitors and sLeX mimetics which can be synthesized by an aldol addition reaction between a glycoside aldehyde precursor and dihydroxyacetone phosphate or a derivative thereof. However, these compounds do not have much selectivity for P-selectin.

Thus there is a need for substances with selective affinity to P-
10 selectin, which can be used for preparing pharmaceutical compositions for the diagnosis, prevention and treatment of various diseases and conditions involving the adherence of leukocytes to vascular endothelial cells or to platelets. There is also a need for P-selectin ligands, which can be used as targeting molecules or moieties in pharmaceutical compositions for the
15 targeting of drugs or genetic material to tissues expressing P-selectin. Furthermore, there is a need for compositions comprising said substances in a form suitable to be used in a more patient friendly way than by parenteral application.

20 OBJECTS OF THE INVENTION

It is therefore an object of the invention to provide compounds with affinity to human P-selectin.

In particular, it is an object of the invention to provide compounds which act as antagonists or partial antagonists of P-selectin.

25 It is another object of the invention to provide compounds which act as targeting ligands with an ability to target drugs and genetic material to cells and tissues expressing P-selectin.

A further object of the invention is the provision of methods for preparing such compounds.

Yet another object is the presentation of uses of such compounds, and of compositions which contain the compounds.

Other objects of the present invention will become clear on the basis of the following description.

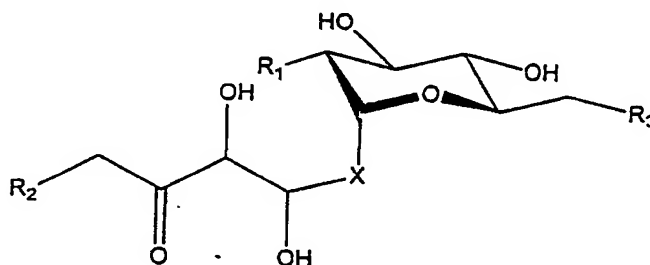
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SUMMARY OF THE INVENTION

According to the invention, glucose-based compounds are provided which have both affinity to and selectivity for P-selectin, and have the potential to be powerful modulators or antagonists of P-selectin. More particularly, the compounds of the present invention are represented by the following formula I:

10

15



wherein:

20 X represents CH_2 , OCH_2 , CH_2OCH_2 , SCH_2 , CH_2SCH_2 , NHCH_2 or CH_2NHCH_2 , preferably CH_2 or OCH_2 ;

R_1 represents QR_4 , wherein Q represents $-\text{O}-$, $-\text{NH}-$, $-\text{NH}-(\text{C}=\text{O})$, $-\text{O}-(\text{C}=\text{O})$, or $-\text{NH}-(\text{C}=\text{O})-\text{NH}$; and wherein R_4 represents H, an aromatic moiety, or a group comprising an electron withdrawing moiety;

25 R_2 is a moiety bearing at least one negative charge; and R_3 can be any group.

In a further aspect, the invention provides methods for preparing the compounds of the invention. The methods optionally include the Fischer synthesis of O-allyl glucopyranosides from glucose, followed by the acetylation

and oxidation to form glycosyl aldehydes, which are subsequently linked to a dihydroxyacetone derivative through an aldolase-catalyzed reaction.

In a further aspect, the invention relates to the compound of the invention as a diagnosticum or a medicine, and especially as antagonist or
5 partial antagonist of P-selectin in the diagnosis, prevention or treatment of diseases and conditions associated with P-selectin.

In yet another aspect, the invention relates to the use of the novel compounds as targeting ligands to direct conjugates or drug carriers to cells and tissues expressing P-selectin. Drug conjugates and drug carriers
10 comprising the novel compounds may be used to specifically deliver active compounds other than the compounds of the invention to such cells and tissues of a mammal.

In yet another aspect, the invention relates to diagnostic and therapeutic compositions comprising the compounds of the invention. The
15 compositions may comprise the compounds or their derivatives, salts, conjugates, or they may comprise a drug carrier system, such as a colloidal or microparticulate system which contains the compounds of the invention.

20

DETAILED DESCRIPTION OF THE INVENTION

The compounds of the invention are derived from the monosaccharide glucose which is modified to have a chemical structure as shown in formula I.

One of the features in which the compounds of the invention differ
25 from known sugar-based sLeX ligands is the type of monosaccharide which is used as a template. The most active selectin ligands known in the prior art are based on fucose or mannose, both having the essential hydroxyl groups, which were previously recognized as important for selectin binding, configured in the same orientation. For instance, one of the most promising lead molecules of the

prior art is structure 2 described in US-A-5,830,871: this compound seems to be a highly active sLeX mimetic, but it does not have selective affinity to P-selectin over E- and L-selectin. According to the present invention, however, the glucose-based derivatives of the invention do have P-selectin specific
5 activity. Choosing modifications through substituents as disclosed herein-below as preferred embodiments, the selective binding to P-selectin is further augmented.

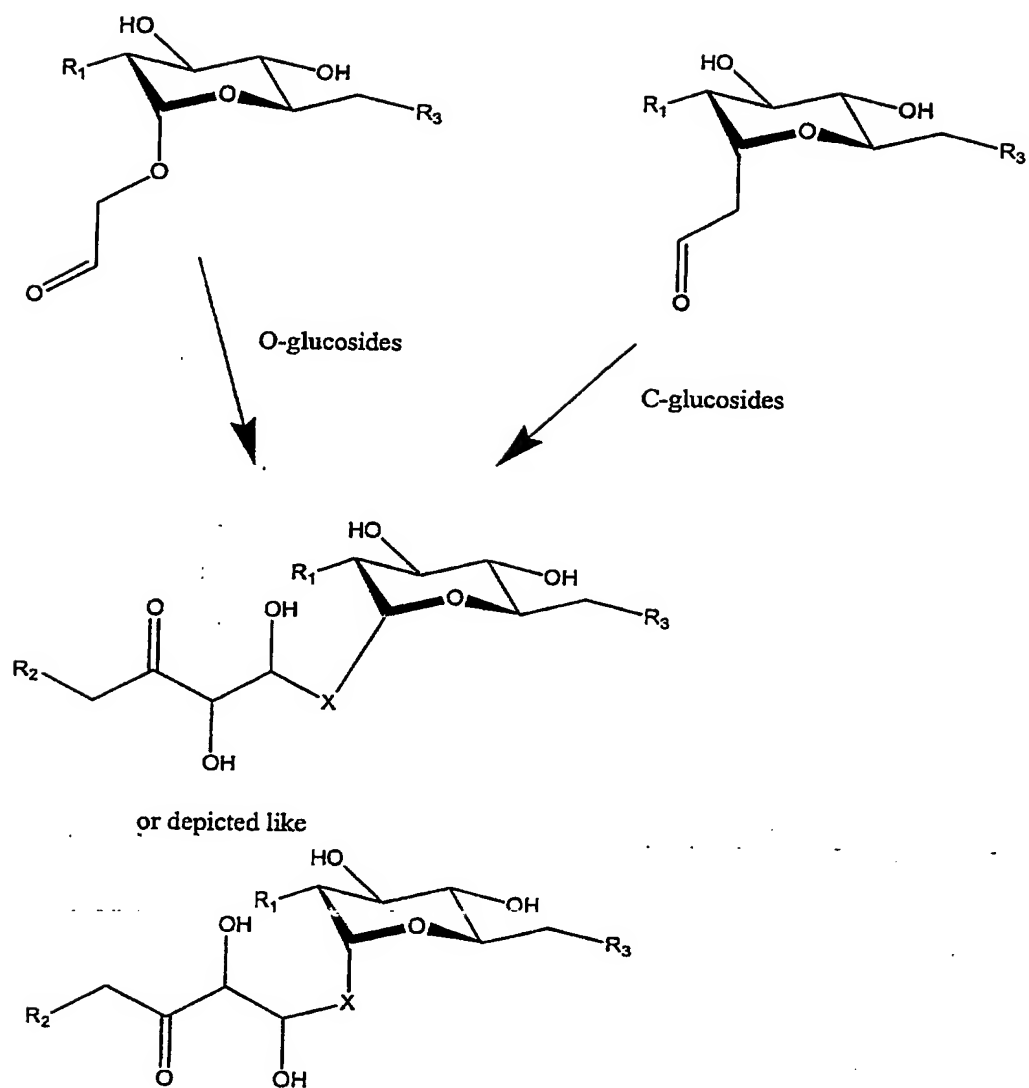
More in detail, previously, fucose- and mannose-based sLeX mimetics were considered the most potent monosaccharide ligands to the
10 selectins (see Wong *et al.*, J. Am. Chem. Soc. 119, 8152-8, (1997)). While this may be true for E- and L-selectin, the present inventors have discovered that glucose-based sLeX mimetics are particularly useful for the selective binding to P-selectin. Unlike E- and L-selectin, P-selectin binding does not require an axially orientated hydroxyl group at the C-2 position of the monosaccharide.
15 This can be shown by the comparison in Figure 1 of the fucose-based lead structure 2 (according to Wong *et al.*, *ibid.*) and the glucose-based analogon, structure 3: with regard to P-selectin, the respective EC₅₀ values are 8.4 mM for compound 2 and 2.0 mM for compound 3, respectively.

The compounds of the invention are further characterized by a 4-
20 oxo-2,3-dihydroxy-n-pentylene group, optionally coupled through a linker group and preferably coupled through an oxygen group (see in this respect, the meaning of X in Formula I), at the C-1 position of the monosaccharide template. This group is further modified by a negatively charged substituent R₂ at its C-5 position. With the term "negatively charged substituent", it is
25 meant a substituent that has a negative charge under physiological compositions. Among the preferred negatively charged substituents are substituents comprising a phosphate group, a phosphonate group, a carboxylate, a sulphonate. Particularly preferred is a phosphate group.

The 4-oxo-2,3-dihydroxy-n-pentyl group is attached to the C-1
30 position of the monosaccharide ringstructure, either directly or through an

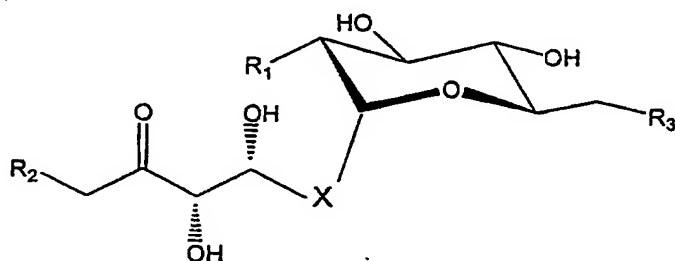
oxygen atom, an OCH_2 group, a sulphur atom, an SCH_2 group, an NH group or an NHCH_2 group, preferably via an oxygen atom. With an eye on formula I, this means that if spacer X is OCH_2 , the oxygen atom of the spacer is directly attached to the sugar, while the carbon atom of the spacer is positioned

5 between the oxygen and the 3-oxo-1,2-dihydroxy-n-butyl group. The two preferred choices of X, *i.e.* CH_2 and OCH_2 , represent the fact that the compounds can be derivatives of C-glucosides or O-glucosides. See in this respect the following reaction scheme:



Without wishing to be limited by any theoretical consideration, it is hypothesized that the compounds of the present invention have a strong interaction with P-selection because the 4-oxo-2,3-dihydroxy-n-pentyl group mimicks the two hydroxyl functions of the galactose moiety and the carboxylic acid function of the N-acetylneuraminic acid within the natural ligand sialyl Lewis X (sLeX).

In a preferred embodiment, the compound of the invention has the stereochemical configuration as shown in formula II:



At the C-6 position of the monosaccharide scaffold, the compounds have a substituent which is herein defined as R_3 . In general, R_3 may represent any substituent, inclusive of a hydrogen atom. In fact, R_3 is the substituent required for the functionalisation. It is not particularly critical what kind of group is present. In one of the preferred embodiments of the invention, R_3 is hydroxyl, which is the group originally present at the C-6 position in the glucose molecule which serves as a monosaccharide template of the compounds. In another preferred embodiment R_3 represents an $-OR_5$ moiety, wherein R_5 comprises at least one carbon atom. Preferred groups R_5 are linear or branched alkyl or aryl groups, linear or branched aralkyl or alkaryl groups, which groups can contain one or more heteroatoms, such as nitrogen, oxygen, phosphorous, sulphur atoms; the groups can be substituted with halogen atoms, hydroxyl groups, oxygen atoms, alkoxy and aryloxy groups, amino or substituted amino groups, as well as other substituents. Instead of through an

oxygen atom, the group R_5 can also directly be coupled to the saccharide core, or through another coupling group by introducing said group in the form of a primary amine, an azide, an aldehyde or carboxylic acid.

Furthermore, the C-6 position is one of the preferred reactive
5 positions where the core structure of the compounds can be coupled to other functional moieties, such as spacers or anchoring moieties. Directly or indirectly via a spacer, the C-6 can be coupled to another active compound which is not derived from a compound of the invention to form a drug
10 conjugate, or it can be coupled to an anchoring moiety which is capable of anchoring the compound to a colloidal or microparticulate drug carrier, such as a liposome, a niosome, a nanodroplet, a nanoparticle, a nanocapsule, a nanosphere, a microparticle, a dendrimer, a microcapsule, a microsphere, a colloidal complex, a micelle, a mixed micelle, a crosslinked micelle, a viral vector, or a lipid complex. As used herein, the anchoring capability comprises
15 both covalent and non-covalent interactions between the anchor moiety and the substrate, *i.e.* the colloidal or microparticulate drug carrier, by which the compound may be linked to the substrate. With respect to these embodiments, R_3 can be formally defined, for instance, as a substituent $-YR_5$ wherein Y is preferably O, CH_2 , or NH, and wherein R_5 is any substituent having at least
20 one carbon atom. In one of the particularly preferred embodiments, Y is oxygen (O).

However, a compound of the invention can also be conjugated to another active compound or moiety (which is not derived from a compound according to the present invention), or to an anchoring moiety, via a different
25 type of linkage, *e.g.* at the C-2 position of the monosaccharide scaffold. In this way, the compound of the invention can be used as a targeting means to direct the other active compound, or the colloidal or microparticulate drug carrier, to cells and tissues expressing P-selectin. Thus it is possible to deliver another
30 active compound specifically to such cells and tissues, regardless of the type of activity or mechanism of action of the other active compound.

In a further embodiment, a compound of the invention is coupled to one or more other compounds or moieties which also bind to P-selectin. For instance, in Applicant's copending international patent application PCT/NL02/00566 and Applicant's copending European patent application no. 02078308.0, compounds with peptidic core regions and certain consensus motifs recognizing P-selectin are disclosed, which compounds can be used in this embodiment. For this purpose, these compounds are incorporated by reference in the present description. Conjugates or colloidal or microparticulate drug carriers comprising at least one compound of the present and at least one peptidic compound with affinity to P-selectin may be particularly useful to provide a high degree of P-selectin binding. This may be assumed because the compounds bind to different sites of the P-selectin molecule: while the peptides have affinity to a non-active binding site, the sugar ligands of the present invention appear to bind to the active site of P-selectin.

The compounds of the invention are further characterized in that they possess a substituent R_1 at the C-2 of the monosaccharide scaffold. This substituent R_1 is much more critical than substituent R_3 . Without wishing to be bound by any theory, it is believed that R_1 plays an active role in the recognition of or selectivity to P-selectin. R_1 represents QR_4 , wherein Q represents $-O-$, $-NH-$, $-NH-(C=O)$, $-O-(C=O)$, or $-NH-(C=O)-NH$; and wherein R_4 represents H, an aromatic moiety, or an electron withdrawing moiety. The aromatic moiety can, for example, be a phenyl, naphthyl, cresyl, tolyl, anthracyl, pyridyl, pyrazyl, pyridazyl or quinolyl group, which group can optionally be substituted. Preferably, R_4 is a phenyl or naphthyl group. In another embodiment, R_4 is a group comprising an electron-withdrawing moiety. Preferably, the electron withdrawing moiety is a moiety selected from the group consisting of nitro, $-(C=O)$ -alkyl, cyanonitrile, $-SO_3H$, CCl_3 or CF_3 ; more preferably, the electron withdrawing group is a nitro group. In a further preferred embodiment, R_1 is $NH(C=O)R_6$, wherein R_6 represents any

substituent comprising at least one carbon atom. Preferred groups R_6 are linear or branched alkyl or aryl groups, linear or branched aralkyl or alkaryl groups, which groups can contain one or more heteroatoms, such as nitrogen, oxygen, phosphorous, sulphur atoms, and which groups preferably have up to 20 carbon atoms, more preferably between 1 and 12 carbon atoms; the groups can be substituted with halogen atoms, hydroxyl groups, oxygen atoms, alkoxy and aryloxy groups, amino or substituted amino groups, as well as other substituents. In especially preferred embodiments, the electron withdrawing groups are present on the aromatic moieties.

The selection of the group R_6 was found to have a significant influence on the P-selectin binding activity of a compound of the invention. Depending on the substituent R_6 , the affinity to P-selectin can actually be increased to reach a several-fold higher affinity than the endogenous ligand sLeX. Surprisingly, it was found that substituents comprising aromatic groups are particularly useful. For instance, the selection of phenyl or naphthalene for R_6 led to compounds with particularly high affinity and selectivity for P-selectin. Without wishing to be bound by any theory, it is assumed that, in the position of R_6 , a substituent that is capable of participating in a π - π interaction may actually be able to bind to a tyrosine group (Tyr⁹⁴) which is present in P-selectin, and which was previously found to be in close proximity to the C-4 hydroxyl group of the galactose moiety of sLeX bound to the CRD domain of P-selectin (Somers *et al.*, Cell, 103, 467-479, 2000). R_6 hence preferably is a substituent able to participate in a π - π interaction with a tyrosine group, and more preferably represents a phenyl or naphthalene group.

Compounds of the invention can be prepared by various different routes of synthesis. Depending on the particular selection of substituents as discussed above, the most useful reaction schemes may differ from each other.

In one of the preferred routes of synthesis, glucose is used as a starting

material to prepare a O-allyl glucopyranoside by Fischer synthesis, which may be followed by the acetylation and oxidation to form glycosyl aldehydes. These

aldehydes are subsequently linked to a dihydroxyacetone derivative through an aldolase-catalyzed reaction.

For the condensation of the glycosyl aldehyde to a dihydroxyacetone phosphate, various types of aldolase can be used as enzymatic catalysts, including D-fructose-1,6-diphosphate aldolase from rabbit muscle (RAMA) and fructose diphosphate aldolase from *St. carnosus* (FruA). As RAMA combines a relatively poor stability in organic solvents with a high substrate specificity, its tolerance to aldehyde acceptors is quite low. In view of its superior solvent stability and substrate specificity, it is therefore recommended to use FruA, which considerably extends the range of conditions and substrates of aldolase catalyzed condensation reactions to be used in organic synthesis. Illustratively, condensation proceeded smoothly even when fully acetylated glycosyl aldehydes and dihydroxyacetone phosphate (DHAP) are used as substrates in a mixture of an organic solvent such as tetrahydrofuran (THF) or dimethylsulfoxide (DMSO) with water.

In order to introduce selected substituents represented by R_1 , again several synthetic pathways can be followed. For instance, if R_1 is selected to be NHR_4 and R_4 chosen to be an acyl group, a glucosamine can be used as starting material. The glucosamine can be converted into the 2,2,2-trichloroethoxycarbonyl (Troc) derivative under Schotten-Baumann conditions and subsequently allylated and acetylated to yield the O-allyl glycoside. After removing the Troc group, the free amino function can be acylated with the respective acyl chlorides.

Salts, adducts, solvates and other derivatives of the compounds as defined above are also within the scope of the invention. In a preferred embodiment, the compound of the invention is in the form of a salt which has been formed by the addition of an acceptable base. However, acid addition salts may also be feasible. Whether an acid or a base is pharmaceutically acceptable can be easily determined by a person skilled in the art taking into consideration the specific intended use. For instance, not all acids and bases

that are acceptable for *in vitro* diagnostic compositions can be used for therapeutic compositions. Depending on the intended use, pharmaceutically acceptable acids include organic and inorganic acids such as formic acid, acetic acid, propionic acid, lactic acid, glycolic acid, oxalic acid, pyruvic acid, succinic acid, maleic acid, malonic acid, cinnamic acid, sulfuric acid, hydrochloric acid, hydrobromic acid, nitric acid, perchloric acid, phosphoric acid, and thiocyanic acid, which form ammonium salts with free amino groups of peptides and functional equivalents. Pharmaceutically acceptable bases, which form carboxylate salts with free carboxylic groups of peptides and functional equivalents, include ethylamine, methylamine, dimethylamine, triethylamine, isopropylamine, diisopropylamine, and other mono-, di- and trialkylamines, as well as arylamines. Moreover, also pharmaceutically acceptable solvates are encompassed.

A further aspect of the invention refers to the uses of the disclosed compounds. Since the compounds bind selectively to P-selectin, they can, depending on their type of interaction with P-selectin after binding, function as antagonists, partial antagonists, or as targeting means to target conjugated substances or drug carrier systems such as liposomes or nanoparticles to cells and tissues expressing P-selectin. Thus, the compounds can be advantageously used in pharmaceutical compositions. According to the invention, such pharmaceutical compositions are provided as well.

As used herein, the term "pharmaceutical composition" refers to therapeutic and diagnostic compositions, as well as to medicaments and diagnostics containing such compositions. Therapeutic compositions and medicaments are used for the prevention, inhibition, reduction or treatment of diseases and other conditions of mammals of which conditions improvement is desired. Diagnostics and diagnostic compositions are used for the diagnosis of such diseases *in vivo* and *in vitro*.

A preferred use of the compounds is for preparing therapeutic compositions or medicaments, especially for diseases or discomforts or other

conditions involving the activation or overexpression of P-selectin, such as therapeutic compositions or medimants to prevent or improve diseases and conditions involving the adhesion of leukocytes, such as monocytes and neutrophils, to the vascular endothelium and to platelets. The compounds can
5 also be used in compositions for treating diseases in which the inhibition of P-selectin-mediated intracellular signaling is desirable.

For instance, compositions containing one or more compounds of the invention can contribute to controlling leukocyte-mediated inflammatory processes. It is known that activated leukocytes release toxic molecules which
10 can damage normal tissue. These inflammatory responses, some of which also involve P-selectin-mediated platelet activation, are part of several pathological conditions, such as (organ) transplant rejection, cold ischemia, hemorrhagic shock, septic shock, tumor metastasis, chronic inflammation, rheumatoid arthritis, inflammatory bowel disease, atherosclerosis, thrombosis, restenosis,
15 angiogenesis, disseminated intravascular coagulation, adult respiratory stress syndrome, circulatory shock, severe traumatic brain injury, relapsing-remitting multiple sclerosis, cerebral artery occlusion, ischemia, stroke, acute myocardial infarct, arterial injury, such as produced by angioplasty, myocardial ischemia, renal injury from ischemia and reperfusion, and renal
20 failure.

In another embodiment, the compounds are used in the preparation of diagnostic compositions or products. Such compositions can be used for *in vitro* tests to quantify P-selectin concentrations in body fluids as markers for the diseases and conditions described above. They may be also used for *in vivo*
25 diagnostic imaging procedures to monitor P-selectin mediated atherosclerosis, aneurisms, restenosis following percutaneous transluminal coronary angioplasty (post-PTCA restenosis), and other conditions selected from those in which P-selectin is mobilized. As an option for this use, a compound according to the invention may be conjugated with a chelator, which is

subsequently complexed with an isotropic label that is detectable by the chosen monitoring system.

Another use of the compounds is as a tool in research. Hence, the present invention also relates to a method for determining whether a
5 compound is capable of binding to P-selectin or a functional equivalent of P-selectin, comprising contacting and incubating the compound to be tested and a predetermined amount of a compound according to the invention with a predetermined amount of P-selectin or said functional equivalent of P-selectin, and subsequently determining the amount of compound according to the
10 invention.

More specific, the compounds of the invention can be used to test the binding affinity of molecules to P-selectin or functional equivalents of P-selectin. To conduct this test method, P-selectin or a functional equivalent of P-selectin is contacted and incubated with a molecule to be tested for binding
15 affinity and with a compound of the invention. A reduced binding of the compound of the invention would indicate an affinity of the molecule to P-selectin.

The compounds can also be used as targeting molecules or conjugates in pharmaceutical compositions for the targeting of drugs or genetic
20 material to tissues that express P-selectin. As conjugates, the compounds can be directly coupled with active molecules or nucleic acids that are to be delivered to such tissues. Alternatively, they can be incorporated into or anchored onto the surface of liposomes or other lipid vesicles, emulsion droplets, polymers, micelles, nano- or microcapsules, nano- or microparticles to
25 obtain targeted vehicles for drugs or genetic material which is delivered to P-selectin expressing tissues.

The pharmaceutical compositions preferably contain one or more compounds with P-selectin affinity as disclosed herein and at least one carrier or excipient. As used herein, a carrier or excipient is any pharmaceutically
30 acceptable substance or mixture of substances having no substantial

pharmacological activity, which can be used as a vehicle or as an auxiliary substance to formulate a compound into dosage form which is stable and easy to administer. Examples of pharmaceutically acceptable excipients are found in the monographs of all major pharmacopoeias.

5 In one embodiment, the composition is formulated and processed for parenteral injection, preferably for intravascular injection, such as intravenous or intra-arterial, but also for intramuscular, subcutaneous, intralesional, intraperitoneal or other routes of parenteral administration. The same principles that govern the formulation of other drugs for these administration
10 routes will also teach those skilled in the art on how to prepare such compositions. For instance, one of the requirements of parenteral dosage forms is their sterility. Other requirements are described in all major pharmacopoeias, such as in USP 24, in the monograph "General Requirements for Tests and Assays. 1. Injections", p. 1775-1777. To increase the stability of a
15 parenteral formulation, it may be necessary to provide a dried dosage form which must be reconstituted before it can be administered. An example of such a dosage form is a freeze-dried or lyophilized formulation.

 It may be desirable to administer a compound of the invention as a parenteral controlled release dosage form to avoid frequent injections and to
20 improve the effectiveness and convenience of the therapy. Various methods of preparing such depot formulations are known. Prolonged release may be provided by solid implants, nanoparticles, nanocapsules, microparticles, microcapsules, emulsions, suspensions, oily solutions, liposomes, or similar structures.

25 Excipients that are particularly useful for the preparation of parenteral formulations are solvents, cosolvents and liquid or semisolid carriers, such as sterile water, ethanol, glycerol, propylene glycol, polyethylene glycol, butanediol, fatty oils, short- and medium chain triglycerides, lecithin, polyoxyethylene castor oil derivatives; substances to adjust the osmolality and
30 pH, such as sugars, especially glucose, sugar alcohols, especially mannitol,

sodium chloride, sodium carbonate, citric acid, acetate, phosphate, phosphoric acid, hydrochloric acid, sodium hydroxide etc.; stabilizers, antioxidants, and preservatives, such as ascorbic acid, sodium sulfite or sodium hydrogen sulfite, EDTA, benzyl alcohol etc.; other excipients and lyophilization aids, such as albumin, dextran etc.

Alternatively, the pharmaceutical compositions may be designed for oral administration and processed accordingly. As a matter of fact, one of the particular advantages of the present invention over other known compounds binding to P-selectin, such as antibodies or peptides, is the fact that they have a higher potential to be stable in gastrointestinal fluids as they are not peptides. Appropriate oral dosage forms include tablets, hard capsules, soft capsules, powders, granules, orally disintegrating dosage forms, syrups, drops, suspensions, effervescent tablets, chewable tablets, oral films, lyophilized dosage forms, sustained release dosage forms, controlled release dosage forms. In one embodiment, the oral dosage form is an enterically coated solid dosage form to provide protection of the compound from the acidic environment of the stomach.

It may also be advantageous to administer a compound of the invention in a transmucosal dosage form. This route of administration is non-invasive and patient-friendly; at the same time it may lead to an improved bioavailability of the compound compared to oral administration, especially if the compound is not stable in the fluids of the digestive system, or if it is too large to be absorbed from the gut effectively. Transmucosal administration is possible via, for instance, nasal, buccal, sublingual, gingival, or vaginal dosage forms. These dosage forms can be prepared by known techniques; they can be formulated to represent nasal drops or sprays, inserts, films, patches, gels, ointments, or tablets. Preferably, the excipients used for a transmucosal dosage form include one or more substances providing for mucoadhesion, thus prolonging the contact time of the dosage form with the site of absorption and thereby potentially increasing the extent of absorption.

In a further embodiment, the compounds are administered via the pulmonary route, using a metered dose inhaler, a nebulizer, an aerosol spray, or a dry powder inhaler. Appropriate formulations can be prepared by known methods and techniques. Transdermal, rectal, or ocular administration may also be feasible in some cases.

It can be advantageous to use advanced drug delivery or targeting methods to deliver a compound of the invention more effectively. For instance, if a non-parenteral route of administration is chosen, an appropriate dosage form may contain a bioavailability enhancing agent, which may be any substance or mixture of substances which increases the availability of the compound. This may be achieved, for instance, by the protection of the compound from degradation, such as by an enzyme inhibitor or an antioxidant. More preferably, the enhancing agent increases the bioavailability of the compound by increasing the permeability of the absorption barrier, which is typically a mucosa. Permeation enhancers can act via various mechanisms; some increase the fluidity of mucosal membranes, while others open or widen the gap junctions between mucosal cells. Still others reduce the viscosity of the mucus covering the mucosal cell layer. Among the preferred bioavailability enhancers are amphiphilic substances such as cholic acid derivatives, phospholipids, ethanol, fatty acids, oleic acid, fatty acid derivatives, EDTA, carbomers, polycarbophil, and chitosan.

EXAMPLES

The invention is further illustrated by the following examples which are not intended to define or limit the scope of the invention.

Example 1: Synthesis of compound 3 (see reaction scheme in figure 2)

Glucose 7 was converted into its O-allyl glucopyranoside through a Fischer synthesis in allyl alcohol in the presence of a catalytic amount of

camphersulfonic acid. The crude mixture was acetylated to yield 8. Compound 8 was oxidized to the corresponding diol by treatment with OsO₄ and N-methylmorpholin N-oxide monohydrate (NMO) in acetone/water and the diol was converted into aldehyde 9 using meta-sodium periodate. The desired α-isomer was obtained after purification by column chromatography. Compound 9 was subsequently linked to dihydroxyacetone phosphate by enzymatic catalysis with fructose diphosphate aldolase from *St. carnosus* (FruA) in a mixture of THF and water (1:3, v/v). Glycosyl phosphate 10 was obtained in good yield after incubation for approximately one day. Due to the presence of the protecting acetyl groups, purification of the product was greatly facilitated and involved flash chromatography. After purification, the acetyl groups were quantitatively removed by addition of a diluted NaOMe solution, resulting in the desired end product 3.

15

Example 2: Binding of compound 3 to P-selectin

The activity of compound 3 was evaluated in an inhibition assay of SO₃-sLe^a-PAA-biotin binding to immobilized P-selectin as described in the literature (G. Weitz-Schmedt *et al.*, Anal. Biochem. 238, 184-190, 1996) and compared to that of its mannosyl analogue (compound 2, see figure 1). More specifically, a 96 microtiter plate (High binding flat bottom, Costar, Corning, U.S.A.) was coated overnight at 4°C with 10 µg/ml goat anti-human IgG in coating buffer (50 mM NaHCO₃, pH 9.6). Subsequently, wells were washed with assay buffer (20 mM HEPES, 150 mM NaCl, 1 mM CaCl₂, pH 7.4) and incubated for 1 hour at 37°C with blocking buffer (3% BSA in assay buffer). After washing with assay buffer, the wells were incubated for 2 hours at 37°C with human P-selectin/IgG-Fc (0.3 µg/ml) or CD4/IgG-Fc (as negative control). Subsequently, wells were washed three times with assay buffer and incubated for 2 hours at 37°C in binding buffer (0.1% BSA, 0.05% Tween 20 in assay

buffer) with 0.33 $\mu\text{g}/\text{ml}$ $\text{HSO}_3\text{-Le}^{\text{a}}$ -PAA-biotin and the compound. The wells were washed six times with binding buffer and 3,3',5,5'-tetramethylbenzidine (TMB)/ H_2O_2 was added and incubated at room temperature for 15 minutes. The reaction was halted by addition of 2M H_2SO_4 and the absorbance was
 5 measured at 450 nm. As determined by this method, the EC_{50} of glucosyl compound 3 for P-selectin was 2.0 mM and thus significantly higher than that of compound 2 (8.2 mM), which is not a compound of the invention.

Example 3: Synthesis of compounds 6d, 6f and 6i (see fig. 1 and reaction
 10 scheme in figure 3)

Glucosamine was converted into the 2,2,2-trichloroethoxycarbonyl (Troc) derivative 14 under Schotten-Baumann conditions. The crude product was allylated in allyl alcohol in the presence of camphersulfonic acid and subsequently acetylated to afford the α -oriented O-allyl glycoside 15d as major
 15 compound. The Troc-group was then removed by incubation with activated zinc in acetic acid, resulting in the intermediate amine 15a. Subsequently, free amine 15a was converted into the amides 15d, 15f and 15i by addition of the following acid chloride under alkaline aqueous conditions: 2,2,2-trichloroethoxycarbonylchloride (for 15d), benzoyl chloride (for 15f), and
 20 naphthoyl chloride (for 15i). The acylated compounds were then subjected to mild oxidation of the O-allyl group with OsO_4 and NMO and subsequently treatment with NaIO_4 . The respective aldehydes were attained in good yield and condensed with DHAP under FruA catalysis as described in example 1 to attain the glucosyl phosphates 17d, 17f, and 17i. The acetyl groups were then
 25 removed by treatment with 10 mM sodium methoxide solution and compounds 6d, 6f, and 6i were isolated.

Compound 6d: ^1H NMR (MeOD): δ 5.03 (d, 1H, $J = 3.6$ Hz, H-1), 4.70 (d, 1H, $J = 12.7$ Hz), 4.53 (d, 1H, $J = 12.7$ Hz), 4.23-4.11 (m, 2H), 3.93-3.85 (m, 3H), 3.77-3.63 (m, 5H), 3.48-3.42 (m, 2H).

Compound 6f: ^1H NMR (MeOD): δ 7.87-7.41 (m, 5H, CH_{arom}), 4.98 (d, 1H, $J = 3.6$ Hz, H-1), 4.69 (d, 1H, $J = 12.7$ Hz), 4.51 (d, 1H, $J = 12.7$ Hz), 4.15 (dd, 2H, $J = 10.8, 3.6$ Hz), 3.86-3.63 (m, 7H), 3.42 (t, 1H, $J = 9.2$ Hz).

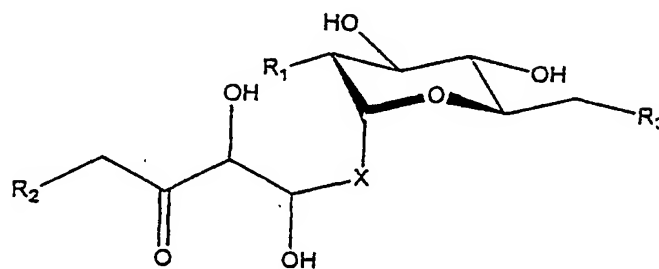
Compound 6i: ^1H NMR (MeOD): δ 7.93-7.55 (m, 7H, CH_{arom}), 4.90-4.61 (m, 4H), 4.02-3.49 (m, 8H).

Example 4: Binding of compounds 6d, 6f, and 6i to P-selectin

The binding of compounds 6d, 6f, and 6i to P-selectin was evaluated using the same inhibition assay as described in example 2. The EC_{50} -values were found to be 3.7 mM for compound 6d, 0.90 mM for compound 6f, and 0.32 mM for compound 6i.

Claims

1. Compound having affinity to and/or selectivity for P-selectin, represented by the following formula I:



wherein:

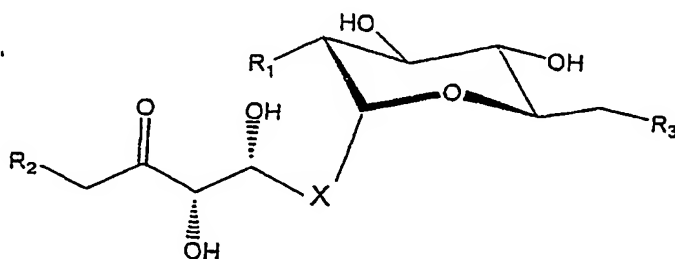
X represents CH_2 , OCH_2 , CH_2OCH_2 , SCH_2 , CH_2SCH_2 , NHCH_2 or CH_2NHCH_2 , preferably CH_2 or OCH_2 ;

15 R_1 represents QR_4 , wherein Q represents $-\text{O}-$, $-\text{NH}-$, $-\text{NH}-(\text{C}=\text{O})$, $-\text{O}-(\text{C}=\text{O})$, or $-\text{NH}-(\text{C}=\text{O})-\text{NH}$; and wherein R_4 represents H, an aromatic moiety, or a group comprising an electron withdrawing moiety;

R_2 is a moiety bearing at least one negative charge; and

R_3 can be any group.

2. The compound of claim 1, represented by the following formula II:



3. The compound of claim 1 or claim 2, wherein X represents OCH_2 .
4. The compound of any one of the preceding claims, wherein R_1 represents NHR_4 .
5. The compound of any one of the preceding claims, wherein R_2 is or comprises a phosphate group.
6. The compound of any one of the preceding claims, wherein R_3 represents OH or OR_5 .
7. The compound of any one of the preceding claims, wherein R_4 is $-(\text{C}=\text{O})\text{R}_6$, wherein R_6 represent a moiety containing at least one carbon atom.
- 10 8. The compound of claim 7, wherein R_6 comprises an aromatic structure.
9. The compound of claim 7 or claim 8, wherein R_6 is a phenyl or naphthalene group.
10. The compound of any one of the preceding claims, wherein R_3 comprises an anchor moiety capable of anchoring the compound to a colloidal or microparticulate drug carrier.
- 15 11. The compound of claim 10, wherein the anchor moiety is a peptide or peptidomimetic moiety having affinity to P-selectin.
12. The compound of any of the preceding claims as a diagnosticum or medicament.
- 20 13. The compound of claim 12 as an antagonist for P-selectin.
14. Use of a compound according to any one of claims 1-13 in the manufacture of a medicament for the diagnosis, prevention or inhibition of a disease or condition involving the activation or overexpression of P-selectin.
- 25 15. Use of a compound according to any one of claims 1-13 as a targeting agent to deliver a pharmaceutically active compound to a cell or to tissue expressing P-selectin.
16. Pharmaceutical composition, comprising in a pharmaceutically
acceptable carrier a compound of any one of claims 1-13, or a derivative, salt,
30 conjugate, solvate, or multimer thereof.

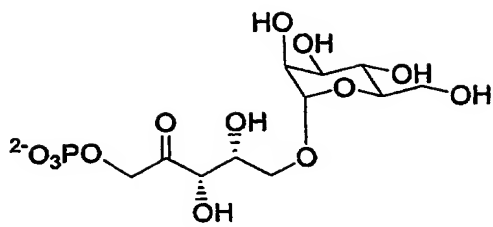
17. A method for determining whether a compound is capable of binding to P-selectin or a functional equivalent of P-selectin, comprising contacting and incubating the compound to be tested and a predetermined amount of a compound according to any one of claims 1-13 with a predetermined amount of
- 5 P-selectin or said functional equivalent of P-selectin, and subsequently determining the amount of compound according to any one of claims 1-13.

Title: Glucose-Based Compounds with Affinity to P-Selectin

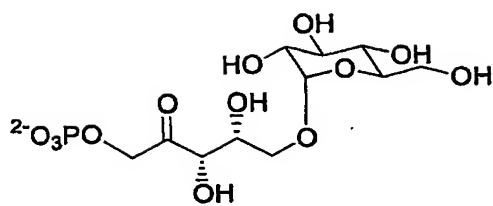
Abstract

The present invention relates to certain glucose-based compounds that have an affinity to P-selectin. Further, the invention relates to the use of these glucose-based compounds in therapeutic or diagnostic methods and in pharmaceutical compositions, to conjugates, pharmaceutical carriers and drug delivery systems comprising said compounds, and to a method for determining whether a compound is capable of binding to P-selectin. In preferred embodiments the glucose-based compounds are used in the manufacture of medicaments or diagnostics for diseases or conditions involving the activation or overexpression of P-selectin.

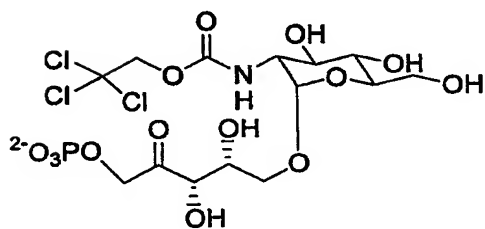
Fig. 1



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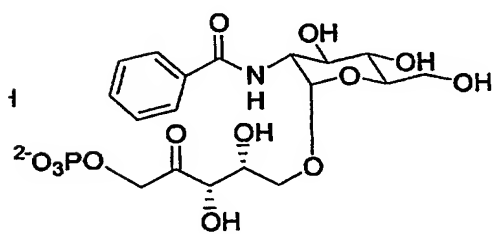


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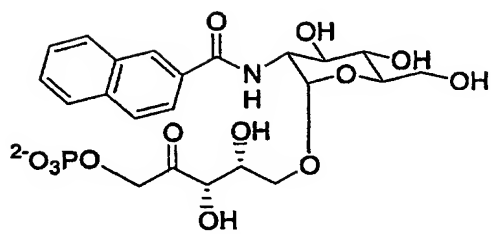


6d

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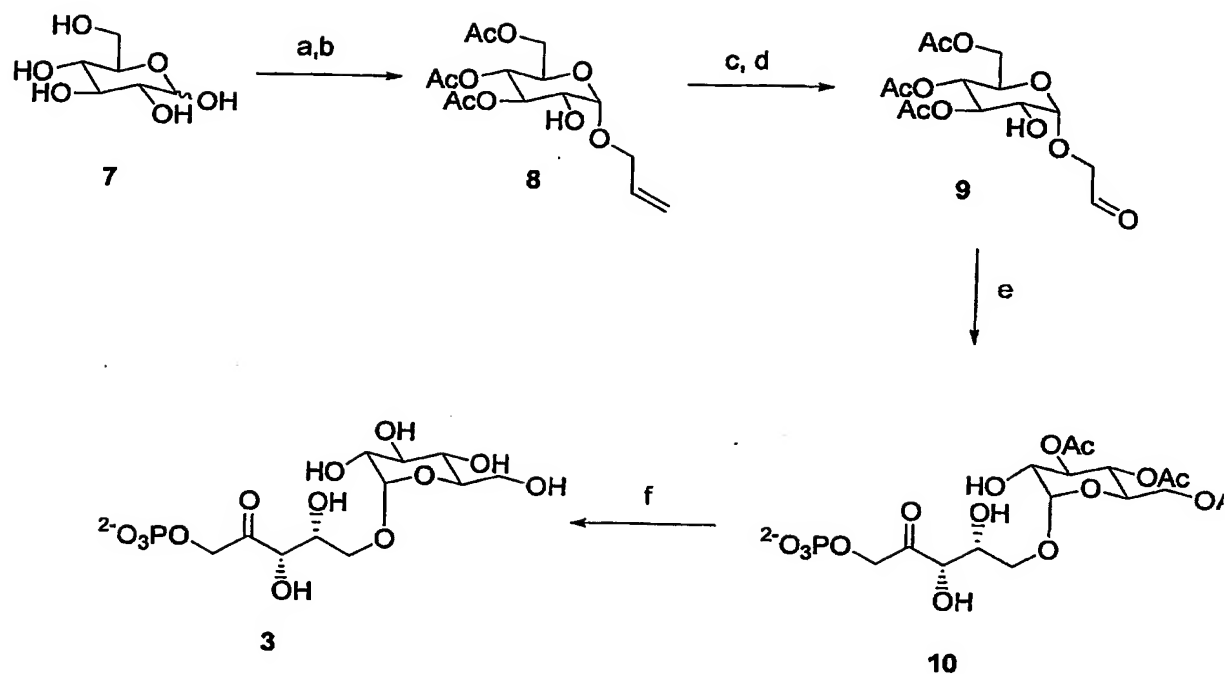


6f



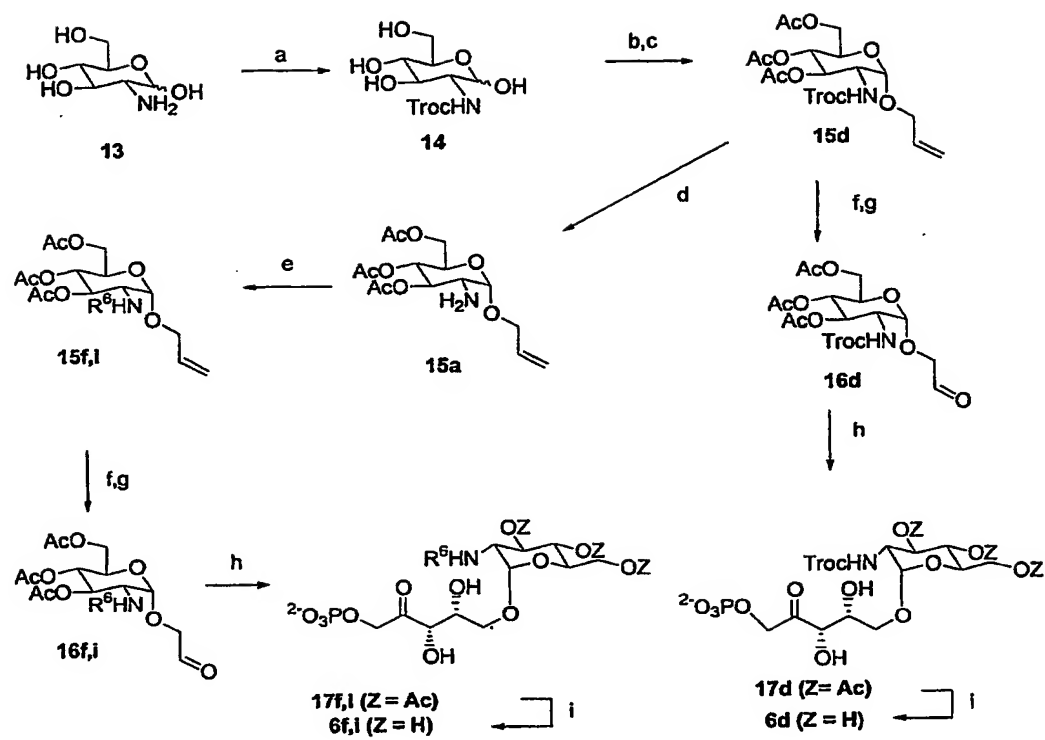
6i

Fig. 2



- 5 Reagents: (a) allyl alcohol, camphersulfonic acid; (b) Ac_2O , pyr, (c) OsO_4 , NMO; (d) NaIO_4 , (e) DHAP, FruA, (f) NaOMe .

Fig. 3



Conditions: (a) Troc-Cl, (b) allyl alcohol, camphersulfonic acid, (c) Ac₂O,
 5 pyridine, (d) zinc, AcOH, (e) R⁶-Cl, (f) OsO₄, NMO, (g) NaIO₄, (h) DHAP, FruA,
 (i) NaOMe.

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